Efficacy of Olyset Duo, a bednet containing pyriproxyfen and permethrin, versus a permethrin-only net against clinical malaria in an area with highly pyrethroid-resistant vectors in rural Burkina Faso: a cluster-randomised controlled trial

Alfred B Tiono, Alphonse Ouédraogo, Daouda Ouattara, Edith C Bougouma, Sam Coulibaly, Amidou Diarra, Brian Faragher, Moussa W Guelbeogo, Nelson Grisales, Issa N Ouédraogo, Zininwindé Amidou Ouédraogo, Margaret Pinder, Souleymane Sanon, Tom Smith, Fiona Vanobberghen, N'Fale Sagnon, Hilary Ranson, Steve W Lindsay

Summary

Background Substantial reductions in malaria incidence in sub-Saharan Africa have been achieved with massive deployment of long-lasting insecticidal nets (LLINs), but pyrethroid resistance threatens control. Burkina Faso is an area with intense malaria transmission and highly pyrethroid-resistant vectors. We assessed the effectiveness of bednets containing permethrin, a pyrethroid, and pyriproxyfen, an insect growth regulator, versus permethrin-only (standard) LLINs against clinical malaria in children younger than 5 years in Banfora, Burkina Faso.

Methods In this two-group, step-wedge, cluster-randomised, controlled, superiority trial, standard LLINs were incrementally replaced with LLINs treated with permethrin plus pyriproxyfen (PPF) in 40 rural clusters in Burkina Faso. In each cluster, 50 children (aged 6 months to 5 years) were followed up by passive case detection for clinical malaria. Cross-sectional surveys were done at the start and the end of the transmission seasons in 2014 and 2015. We did monthly collections from indoor light traps to estimate vector densities. Primary endpoints were the incidence of clinical malaria, measured by passive case detection, and the entomological inoculation rate. Analyses were adjusted for clustering and for month and health centre. This trial is registered as ISRCTN21853394.

Findings 1980 children were enrolled in the cohort in 2014 and 2157 in 2015. At the end of the study, more than 99% of children slept under a bednet. The incidence of clinical malaria was 2·0 episodes per child-year in the standard LLIN group and 1·5 episodes per child-year in the PPF-treated LLIN group (incidence rate ratio 0·88 [95% CI 0·77–0·99; p=0·04]). The entomological inoculation rate was 85 (95% CI 63–108) infective bites per transmission season in the standard LLIN group versus 42 (32–52) infective bites per transmission season in the PPF-treated LLIN group (rate ratio 0·49, 95% CI 0·32–0·66; p<0·0001).

Interpretation PPF-treated LLINs provide greater protection against clinical malaria than do standard LLINs and could be used as an alternative to standard LLINs in areas with intense transmission of *Plasmodium falciparum* malaria and highly pyrethroid-resistant vectors.

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Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso (A B Tiono PhD, A Ouédraogo PhD, D Ouattara MD, E C Bougouma PhD, S Coulibaly MD, A Diarra PhD, M W Guelbeogo PhD, I N Ouédraogo PhD, Z A Ouédraogo MSc, S Sanon PhD, N'F Sagnon PhD); **Department of Biosciences, Durham University, Durham, UK** (M Pinder PhD, Prof S W Lindsay PhD); **Liverpool School of Tropical Medicine, Liverpool, UK** (Prof B Faragher PhD, N Grisales PhD, Prof H Ranson PhD); **Swiss Tropical and Public Health Institute, Basel, Switzerland** (F Vanobberghen PhD, Prof T Smith PhD); **and University of Basel, Basel, Switzerland** (F Vanobberghen, Prof T Smith)

Correspondence to:
Prof Steve W Lindsay, Department of Biosciences, Durham University, Durham DH1 3LE, UK
s.w.lindsay@durham.ac.uk

Introduction

Malaria control in sub-Saharan Africa has improved considerably, with malaria prevalence decreasing by roughly 50%, and the incidence of clinical disease decreasing by 40%, from 2000 to 2015.[1] This reduction was achieved largely by massive scaling-up of long-lasting insecticidal nets (LLINs) and indoor residual spraying. Yet, according to WHO,[2] the decline in malaria incidence and mortality has stalled, and they are even increasing in some countries. Burkina Faso, which has more than 6 million cases of malaria in children younger than 5 years,[3] is one of 20 countries in sub-Saharan African where malaria cases increased between 2015 and 2016. It is also one of the few countries with no significant association between LLIN ownership and reduction in child mortality.[4] Although about 90% of households in Burkina Faso owned at least one LLIN in 2013,[5,6] national surveys found that they had little effect on *Plasmodium falciparum* prevalence, with 61% of children aged 6 months to 5 years infected in 2014.[6] One possible reason for this ineffectiveness is that malaria parasite vectors in Burkina Faso,[7] and across most of sub-Saharan Africa,[8] are becoming increasingly resistant to the pyrethroid insecticides used to treat LLINs.

Formulations are under development for treatment of LLINs consisting of a pyrethroid in combination with another active ingredient, such as pyriproxyfen, to increase their effectiveness.[9–11] Pyriproxyfen is an insect-growth regulator recommended for vector control by WHO because of its effectiveness at extremely low

Research in context

Evidence before this study

We searched MEDLINE and the Cochrane Infectious Diseases Group Specialized Register using the term "malaria" and one or more of the terms "pyriproxyfen", "combination nets", "long-lasting insecticidal nets", "insecticide treated nets", "malaria control", and "vector control" for randomised controlled trials, controlled before-and-after intervention studies, and interrupted time-series analyses of trials of long-lasting insecticidal nets (LLINs) published between Jan 1, 2003, and Dec 31, 2017. We restricted our search to 2003 onwards because the Cochrane review of insecticide-treated bednets and curtains for prevention of malaria covered up to 2003 and did not identify any controlled trials comparing pyriproxyfen-treated nets with conventional LLINs in control of malaria, nor any trials testing mixtures of active ingredients with different modes of action. The only trial to test a mixture of chemicals was published after our search had been completed and used a net containing permethrin and the synergist piperonyl butoxide. The mixture net provided better protection against malaria than did conventional LLINs. Piperonyl butoxide potentiates the insecticidal activity of permethrin, but is not in itself insecticidal and cannot fully restore susceptibility of all resistant populations.

Added value of this study

To our knowledge, this clinical trial is the first to assess the effectiveness of a bednet treated with pyriproxyfen and of a bednet containing a mixture of two active ingredients with different modes of action. Use of mixtures of active ingredients is considered to be superior to use of single active ingredients for management of insecticide-resistant vectors because development of resistance to two or more active chemicals with different modes of action is likely to be considerably slower than for a single active ingredient. We found that the net containing a mixture of pyriproxyfen, an insect growth regulator, and permethrin, a pyrethroid insecticide, provided better protection against clinical malaria in children than did a net treated with only permethrin in an area in Burkina Faso where malaria mosquitoes are highly resistant to pyrethroids. The new net worked by reducing the vector population density and adult lifespan, thereby reducing the number of infective bites.

Implications of all the available evidence

Pyrethroid-resistant vectors are now common throughout sub-Saharan Africa, making control of malaria with pyrethroid-only nets challenging. Nets containing a mixture of active ingredients, such as Olyset Duo, provide better protection than do conventional pyrethroid-only nets in the prevention of clinical malaria in areas of intense transmission of malaria via pyrethroid-resistant vectors.

concentrations, safety in humans,[12] and different mode of action to other classes of insecticides used for vector control. Pyriproxyfen is primarily used as a larvicide, preventing metamorphosis of pupae into adults, and can be effective for 5–9 months after initial treatment.[13,14] It can also sterilise adult mosquitoes, or at least reduce their fecundity and longevity.[15–17]

See Online for appendix

In experimental hut trials, polyethylene nets treated with a combination of permethrin and pyriproxyfen (PPF; trade name Olyset Duo; Sumitomo Chemical, Tokyo, Japan) were associated with increased mortality and reduced blood feeding in pyrethroid-resistant *Anopheles gambiae* sensu stricto compared with permethrin-only LLINs (trade name Olyset).[10,18] Furthermore, PPF-treated LLINs sterilised blood-fed mosquitoes that survived exposure to pyrethroids.[9,18] Similar findings were observed in a pilot study[11] in which PPF-treated LLINs were introduced into village houses in Kenya. We aimed to assess whether PPF-treated LLINs provide superior protection over permethrin-only (standard) LLINs against clinical malaria in children younger than 5 years in Burkina Faso.

Methods

Study design and participants

A detailed description of the study protocol has been reported previously.[19] This study was a two-group, step-wedge, cluster-randomised, controlled, superiority trial done in 2014–15 in the southeast of Banfora, Burkina Faso, a country where malaria is highly endemic.[20] Resistance to permethrin in the study site was high, with discriminating dose assays designed to kill 100% of susceptible mosquitoes killing only 13·4–19·2% of *A gambiae* sensu lato in 2014 and 1·0–19·8% in 2015 (appendix).

Before any study activity, village-level permission was sought after sensitisation meetings attended by village community leaders and local health staff. Consenting villages were grouped into 40 clusters, each consisting of one to four neighbouring villages. A census of the study villages was done in 2013 to produce a list of all children fulfilling the age criteria (age 6 months to 5 years). From this list, children were randomly selected for enrolment in the cross-sectional survey. We did four cross-sectional surveys. The first survey was done at the start of the transmission season in June to July, 2014, and defined the cohort of children enrolled in the study, with an average of 50 children per cluster and roughly equal numbers of children aged 6–35 months and 36–60 months. A second survey was done at the end of the first year of the study in December, 2014, when equal numbers of clusters were in each of the study groups. This survey included the cohort children and at least an additional 50 randomly selected children per cluster (stratified by age). At the third survey at the beginning of the

transmission season in May to July, 2015, children who had dropped out of the study were replaced, when possible, by children of a similar age, and at least 50 additional randomly selected children per cluster were also included. A fourth and final survey was done at the end of the study in December, 2015, in the cohort of children and in at least an additional 50 randomly selected children per cluster.

This study was done in accordance with the principles established in the International Conference on Harmonisation Tripartite Guideline for Good Clinical Practice and the Declaration of Helsinki, whichever afforded the greater protection to the participants. The trial was approved by the Ethics Committee for Health Research, Burkina Faso, on May 13, 2014 (reference 2014-3-24), and by the School of Biological and Biomedical Sciences Ethics Committee, Durham University, UK, on Jan 17, 2014. A Data Safety Monitoring Board reviewed the trial procedures and results. The only incentives given to households that participated in the trial were provision of LLINs, treatment of study children during the study, and fares to reach referral clinics (refunded by study staff on the basis of known tariffs). Written informed consent was obtained from each net recipient, before net donation and exchange. An additional signed informed consent was required from parents or legally acceptable representatives of children who participated in the clinical assessments.

Randomisation and masking

At the start of the malaria transmission season in June, 2014, five randomly selected clusters of villages were provided with PPF-treated LLINs and the remaining 35 village clusters with standard LLINs (figures 1 and 2; appendix). Five clusters were then randomly selected each month from July, 2014, to September, 2014, for replacement of standard LLINs with PPF-treated LLINs, so that, by the end of 2014, each study group had an equal number of clusters (figure 1). In 2015, PPF-treated LLINs were deployed in a similar fashion from June to September. We used this step-wedge design because it represents the type of deployment used by net distribution programmes. Random selection was done with Stata version 10.

Observer bias was reduced when feasible: both types of nets were of similar shape, size, and colour, and blood films were read by microscopists masked to the identity and intervention status of the participants.

Procedures

White nets (1·8 m wide by 1·9 m long by 1·5 m high) containing 2% weight for weight (w/w) permethrin incorporated into polyethylene fibres (Olyset Net; Sumitomo Chemical) were distributed to achieve one LLIN per bed or sleeping place at the beginning of the transmission season in 2014. PPF-treated LLINs were the same size as standard LLINs and contained 2% w/w permethrin and 1% w/w pyriproxyfen incorporated into polyethylene fibres (Olyset Duo; Sumitomo Chemical). The chemical content of 30 randomly selected LLINs and 30 PPF-treated LLINs was checked with high-performance liquid chromatography at the Liverpool School of Tropical Medicine (Liverpool, UK), which confirmed the target doses (appendix). PPF-treated LLINs were stored and distributed in a similar manner to the standard LLINs. PPF-treated LLINs were exchanged for standard LLINs at the household level. Burkina Faso Government's Roll Back Malaria information, education, and communication procedures were followed to encourage correct net use and maintenance for both type of nets.

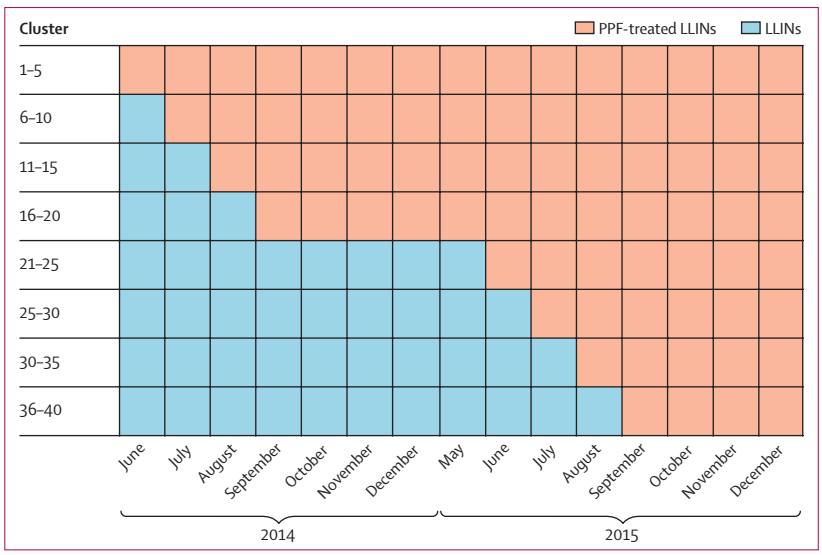

Figure 1: **Distribution of interventions throughout the study**
PPF=permethrin and pyriproxyfen. LLIN=long-lasting insecticidal net.

Passive case detection was used to measure clinical malaria incidence in the cohort children, taking into account time at risk. Parents or carers of cohort children were encouraged to take their child to the nearest local health facility if the child was unwell. Clinical malaria was defined as a child presenting at a government health clinic with an axillary temperature of 37·5°C or higher, or a history of fever in the past 48 h, together with the presence of *P falciparum* parasites of any density detected by the Paracheck-Pf rapid diagnostic test (RDT; Orchid Biomedical Systems, Goa, India) in the absence of other detectable causes of fever. To standardise data collection in the six health centres, we posted one trained study nurse to each facility. These nurses diagnosed malaria using RDTs and prepared blood slides that were subsequently read at the Centre National de Recherche et de Formation sur le Paludisme laboratory in Banfora. These nurses also communicated regularly with their managers to prevent stock-outs.

To facilitate documentation of all consultations with study children, all enrolled children were issued with enumerated photo identity cards. A child's study number,

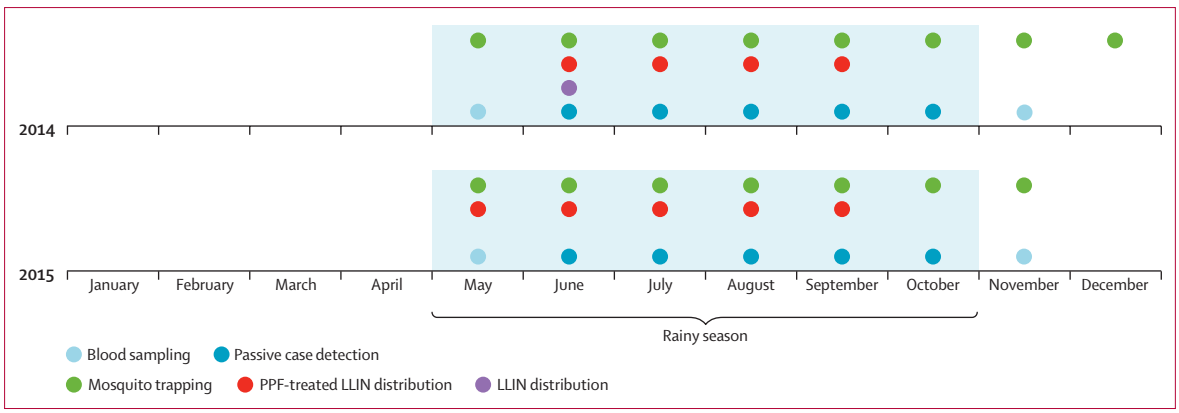

Figure 2: **Timings of interventions and outcome assessment**
PPF=permethrin and pyriproxyfen. LLIN=long-lasting insecticidal net.

initials, and village code were recorded for all clinical events on the case report form. We reimbursed the travel costs for patients visiting the health centres to encourage uptake of the intervention and reporting to the clinics when ill.

We also used anonymised medical records from three health facilities located outside of the main study area to document clinical malaria based on positive RDTs in children aged 6 months to 5 years who lived in villages outside of the study area. Although bednets were not provided by the study team to this area during the study, they were distributed during a nationwide bednet distribution campaign in 2014. Data from these records allowed tracking of temporal changes in malaria incidence in the absence of the intervention, caused by factors such as weather patterns. Comparing this trend with that in the study area provided additional evidence of the scale of community-wide intervention effects.

During the four cross-sectional surveys, all children were visited at home and examined clinically for obvious symptoms and signs of illness and body temperature. Finger-prick samples were collected for haemoglobin measurement with a portable spectrophotometer (HemoCue version 2.1; HemoCue AB, Ängelholm, Sweden) and for thick blood films. Children with fever (axillary temperature ≥37·5°C) or fever in the past 48 h were tested for malaria with an RDT and, if positive, referred to the nearest health facility.

Thick blood films were stained with Giemsa and examined under ×1000 magnification. Parasite counts were recorded per high-power field, and 100 fields were counted before a slide was declared negative. Parasite density was estimated assuming that one parasite per high-power field was equal to 500 parasites per µL. Two slides were prepared from each individual and the best slide assessed separately by two experienced microscopists, with discrepancies resolved by a third microscopist.

We assessed exposure to malaria-vector mosquitoes using Centers for Disease Control and Prevention light traps every 4 weeks from May, 2014, to December, 2014, and from May, 2015, to November, 2015, in six randomly selected households from each cluster (figure 2). Light traps were positioned next to a single sleeper protected with a standard LLIN or a PPF-treated LLIN. Mosquitoes were identified by microscopy and the numbers of *A gambiae* sensu lato and other anophelines recorded. Sporozoites were detected with an ELISA,[21] and a randomly selected subset of female *A gambiae* sensu lato mosquitoes were typed to species by PCR.[22] Parity of mosquitoes was assessed by dissection.

Outcomes

Primary endpoints were the incidence of clinical episodes of malaria among cohort children presenting at health facilities (clinical primary endpoint) and the entomological inoculation rate (entomological primary endpoint).[23] Secondary endpoints, measured at the end of the transmission season in 2014 (when equal numbers of clusters were in each of the study groups), were presence of malaria parasites, prevalence of high parasitaemia (≥5000 parasites per µL), haemoglobin concentration, and prevalence of moderate (haemoglobin <80 g/L) or severe (haemoglobin <50 g/L) anaemia. Children in the cohort were visited at home once a month by project staff for the duration of the passive case detection, and if a child was absent for more than 50% of a given calendar month then their data were censored for that month.

We recorded adverse events and serious adverse events in the study cohort and population during the study. The trial followed standard definitions for adverse events and serious adverse events agreed by consensus of the Collaborating Centres of the WHO International Drug Monitoring Centre (Uppsala, Sweden). The occurrence of adverse events was ascertained by non-directive questioning of the study children at monthly visits during the trial. Adverse events were also recorded if volunteered by study children, parents, or carers, or if noted by nurses through physical examination, laboratory tests, or other assessments at any contact with the participant.

Furthermore, we recorded pregnancy outcomes for all pregnant women in the study area and asthma signs or symptoms in pre-identified residents with asthma.

Statistical analysis

Statistical analyses were done with Stata version 14. For the power calculation, we assumed an incidence of clinical episodes of malaria of 1·5 episodes per child each year and a coefficient of variation of 0·5.[19] The study was designed to detect a protective efficacy of 25% with PPF-treated LLINs compared with standard LLINs, with 90% power and at a 5% significance level. For the entomological endpoint, the study was 80% powered to detect a 33% reduction at a 5% significant level. Methods for sample size calculation have been described previously.[19]

The statistical analysis followed the statistical analysis plan written before completion of the trial. The analysis of the primary clinical endpoint included all children who had a clinical episode of malaria, excluding children who had an episode in the month of or after the introduction of the intervention. For other outcomes, we excluded data from clusters the month of (but not after) the introduction of the PPF-treated LLIN. In cases of malaria, further attacks of malaria in the 4 weeks after treatment were censored because attacks within this period might have been false positives resulting from non-malaria fever and a positive RDT response due to persistence of parasite antigen, and not an active infection.

We calculated the number of clinical episodes, child-years at risk, and the incidence rate ratio (calculated as the ratio of the number of clinical episodes to child-years at risk) for each month of the trial in each group, and ascertained the intervention effectiveness by subtracting the relative rate from the number 1·00. Confidence intervals for effectiveness estimates were obtained with approximations developed by Bennett and colleagues.[24] We used Poisson regression models with log-transformed time at risk as an offset, and with inclusion of village cluster as a random effect and calendar month and health facility as fixed effects. We considered adjusting for potential confounders (age, when participant joined the cohort, coverage, and cluster size), and incorporated an

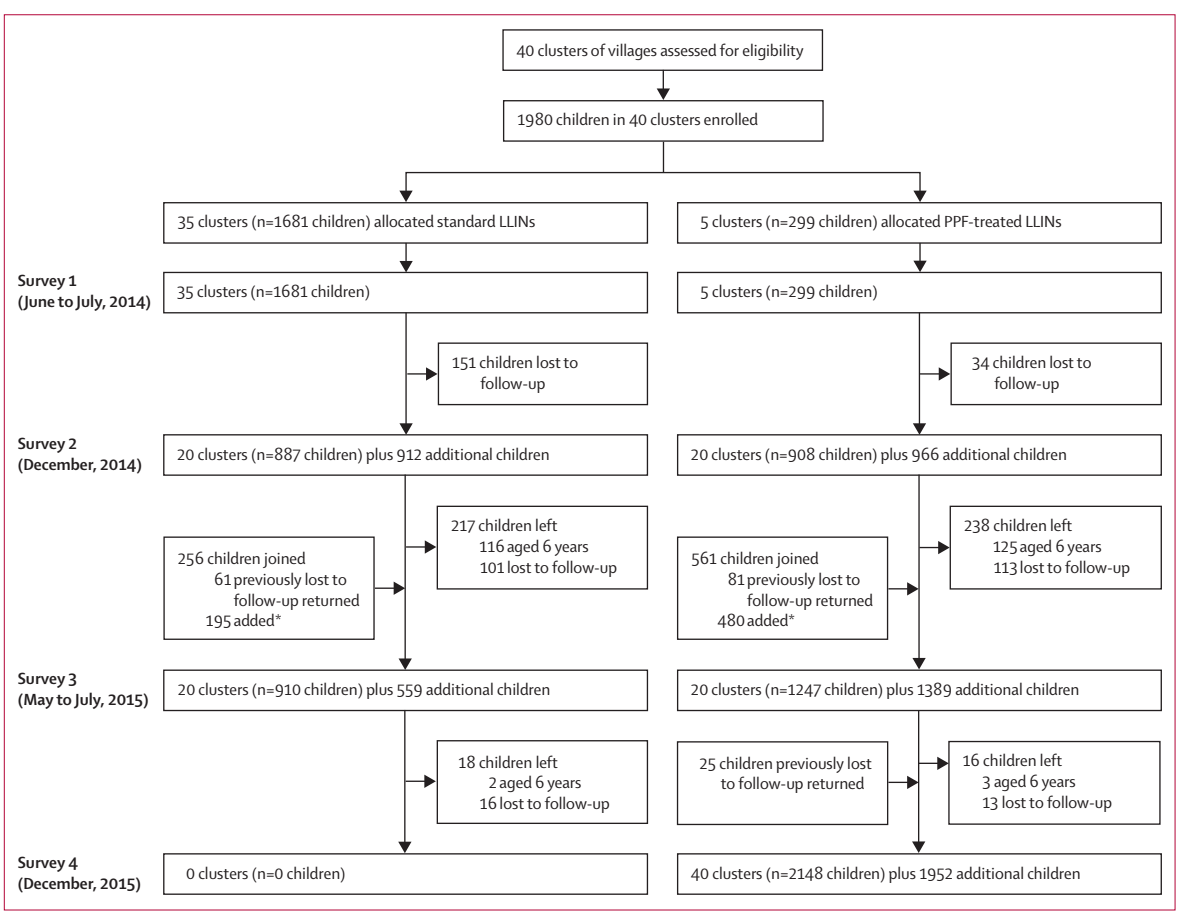

Figure 3: **Trial profile**

The additional children did not form part of the cohort and were, therefore, not included in the passive case detection for the primary endpoint; they were randomly selected from the clusters (at least 50 children from each cluster) for inclusion in the surveys (and so contributed to secondary endpoints). Children who had recently received PPF-treated LLINs were excluded from some of the analyses. LLIN=long-lasting insecticidal net. PPF=permethrin and pyriproxyfen. *675 children were added to the cohort at the third survey to replace those who had exited or were lost to follow-up.

	June, 2014	July, 2014	August, 2014	September, 2014	June, 2015	July, 2015	August, 2015	September, 2015	Total
Children enrolled	274	215	270	256	216	220	251	278	1980
Girls	118 (43%)	105 (49%)	126 (47%)	131 (51%)	108 (50%)	99 (45%)	130 (52%)	145 (52%)	962 (49%)
Boys	156 (57%)	110 (51%)	144 (53%)	125 (49%)	108 (50%)	121 (55%)	121 (48%)	133 (48%)	1018 (51%)
Age (months)	34 (22–48)	37 (21–48)	35 (22–50)	34 (21–49)	35 (23–49)	35 (23–49)	35 (22–47)	35 (22–48)	35 (22–48)
Sleeps under a mosquito net	254 (93%)	184 (86%)	261 (97%)	238 (93%)	209 (97%)	197 (90%)	217 (86%)	268 (96%)	1828 (92%)
Received antimalarials in past 14 days	21 (8%)	13 (6%)	12 (4%)	26 (10%)	11 (5%)	15 (7%)	28 (11%)	17 (6%)	143 (7%)
Sick with a fever during past 48 h	42 (15%)	24 (11%)	35 (13%)	29 (11%)	19 (9%)	33 (15%)	33 (13%)	35 (13%)	250 (13%)
Axillary temperature (°C)	36·7 (36·4–37·1)	36·6 (36·4–36·9)	36·7 (36·4–36·9)	36·7 (36·5–36·9)	36·6 (36·2–36·9)	36·6 (36·2–36·9)	36·4 (36·2–36·9)	36·5 (36·1–36·9)	36·6 (36·3–36·0)
Positive rapid diagnostic test	38/52 (73%)	16/28 (57%)	32/50 (64%)	17/38 (45%)	16/20 (80%)	38/49 (78%)	26/36 (72%)	43/48 (90%)	226/321 (70%)
Presence of *Plasmodium falciparum* parasites by microscopy	120/273 (44%)	93/182 (51%)	141/267 (53%)	138/249 (55%)	96/207 (46%)	114/220 (52%)	141/248 (57%)	138/272 (51%)	981/1918 (51%)
>5000 *P falciparum* parasites per µL	36/273 (13%)	26/182 (14%)	31/267 (12%)	36/249 (14%)	35/207 (17%)	34/220 (15%)	43/248 (17%)	30/272 (11%)	271/1918 (14%)
P falciparum parasite density (per µL)*	1475 (7·2)	1988 (5·0)	1378 (5·5)	1697 (6·3)	2479 (6·6)	1823 (6·9)	1784 (6·1)	1474 (5·3)	1698 (6·1)
Presence of *P falciparum* gametocytes	49/273 (18%)	33/182 (18%)	64/267 (24%)	51/249 (20%)	42/207 (20%)	38/220 (17%)	54/248 (22%)	49/272 (18%)	380/1918 (20%)
Haemoglobin (g/L)	103·0 (91·0–113·0)	101·0 (91·0–110·0)	103·0 (94·0–111·0)	107·0 (101·0–113·0)	103·0 (94·5–115·0)	98·0 (91·0–109·0)	101·0 (96·0–107·0)	102·0 (94·0–111·0)	102·0 (94·0–1110·0)
Moderate anaemia†	27/266 (10%)	20/209 (10%)	22/270 (8%)	8/241 (3%)	11/156 (7%)	19/220 (9%)	8/173 (5%)	16/265 (6%)	131/1800 (7%)
Severe anaemia‡	0/266 (0%)	0/209 (0%)	0/270 (0%)	0/241 (0%)	1/156 (1%)	0/220 (0%)	1/173 (1%)	0/265 (0%)	2/1800 (0%)

Data are n (%), median (IQR), or n/N (%), unless otherwise specified. PPF=permethrin and pyriproxyfen. LLIN=long-lasting insecticidal net. *Data are geometric mean (geometric SD) of non-zero values. †Moderate anaemia was defined as haemoglobin <80 g/L. ‡Severe anaemia was defined as haemoglobin <50 g/L.

Table 1: **Characteristics of children enrolled in the cohort at the first survey (June to July, 2014), by month of rollout of PPF-treated LLINs**

interaction term between study group and calendar month to provide month-specific estimates. These findings should be interpreted cautiously because interaction tests are typically underpowered.[25] We explored how changing the threshold of parasite density might affect the effect estimates of the intervention (appendix).

Secondary endpoints were reported by group with means and SDs for continuous outcomes and numbers and percentages for categorical outcomes. Continuous outcomes were compared between groups with linear regression models and categorical outcomes with logistic regression models, with village cluster as a random effect and health facility as a fixed effect. We repeated the analyses stratified by age (<30 months *vs* ≥30 months; defined a priori) and by whether participants were included in the original cohort or were added later (defined post-hoc).

Numbers of indoor mosquitoes, proportions of mosquitoes with sporozoites or that were parous, and species composition were summarised by group and compared with logistic or negative binomial regression models, with inclusion of village cluster as a random effect and month and health facility as fixed effects. The entomological inoculation rate was defined as the number of infective bites received per person during the transmission season and was calculated as the household density of mosquitoes (estimated as the mean number of *A gambiae* sensu lato per trap each night) multiplied by the proportion of mosquitoes with sporozoites and by the number of days in the transmission season (n=214 for May to November; appendix). We excluded light-trap collections for the calendar month that PPF-treated LLINs were introduced into a village to allow for a delay in the effect of the intervention on the vector population.

Role of the funding source

The funder of the study and net manufacturers had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

In 2013, we approached 92 villages in Banfora, Burkina Faso, of which 91 agreed to participate in this study after community-level meetings to discuss the nature of the study and what would be required during the interventions and investigations. In June, 2014, we distributed 29 084 LLINs (24 357 standard and 4727 PPF treated) to cover the 30 608 sleeping places identified during the pre-study population census, yielding an overall coverage of 95%. Overall, coverage exceeded 80% in all clusters except for one in which residents' multiple absences from home for traditional gold-mining activities made it impossible to deliver nets despite numerous attempts (appendix).

Figure 3 illustrates the flow of the cohort children through the trial, with some being lost (and some returning) and some exiting the study on reaching age 6 years. At baseline, 1980 children were enrolled into 40 clusters; the baseline characteristics of these children were similar across clusters (grouped by month of rollout of PPF-treated LLINs), although there was large variation in RDT positivity between clusters (table 1). 675 children were added at the third survey to replace those who were lost to follow-up or had dropped out of the study; these children had largely similar characteristics to those already enrolled (appendix). By the fourth survey at the end of study, the cohort comprised 2148 children. A mean of 46–56 additional children per cluster were included in surveys two to four.

The overall incidence of clinical malaria was 1·5 per child-year at risk in the PPF-treated LLIN group versus 2·0 per child-year at risk in the LLIN group (figure 4, table 2), corresponding to a crude relative risk of 0·76 (95% CI 0·71–0·81; table 2). This estimate of the effect size is biased because, in both 2014 and 2015, incidence was higher in the first part of the transmission season (when more children were in the LLIN group) than in the September to December period (when more children were in the PPF-treated LLIN group; figure 4). Various analyses adjusting for calendar month gave lower estimates of the reduction in incidence (appendix), with the prespecified primary analysis that included adjustment for month and health facility providing evidence of a significant difference between the groups (incidence rate ratio 0·88, 95% CI 0·77–0·99; p=0·04; table 2). Health facility and calendar month were significantly associated with clinical incidence (p=0·0001 and p<0·0001, respectively), whereas there was no evidence of an interaction by month (p=0·97).

Similar results for the primary endpoint were obtained when adjusting for potential confounders (appendix). The number of malaria cases reported outside of the study area was similar in 2014 and 2015, indicating no change in the intensity of malaria transmission between the two years (appendix). The use of a more specific case definition did not lead to a higher effectiveness estimate (appendix).

At the second survey, the proportion of children with *P falciparum* infection was not significantly different between the groups (odds ratio [OR] 0·93, 95% CI 0·74 to 1·15; p=0·50; table 3). High parasitaemia (>5000 *P falciparum* parasites per µL) was non-significantly decreased (0·87, 0·73 to 1·02; p=0·09; table 3) and haemoglobin concentration was non-significantly increased (coefficient 1·8 g/L, 95% CI –0·4 to 3·9; p=0·11; table 3) with PPF-treated LLINs compared with standard LLINs. However, haemoglobin concentrations were significantly increased with PPF-treated LLINs in children younger than 30 months (99·3 g/L *vs* 95·4 g/L for those younger than 30 months who received standard LLINs; coefficient 3·5 g/L, 95% CI 0·9 to 6·1; p=0·008; appendix). The prevalence of moderate anaemia was lower in the PPF-treated LLIN group than in the standard LLIN group (OR 0·48, 95% CI 0·24–0·96; p=0·04; the difference between the groups could not be estimated for severe anaemia). The proportion of additional (non-cohort) children with high parasitaemia was lower in the PPF-treated LLINs group than in the standard LLINs group (20% *vs* 24%; 0·80, 0·61–0·96; p=0·02; appendix).

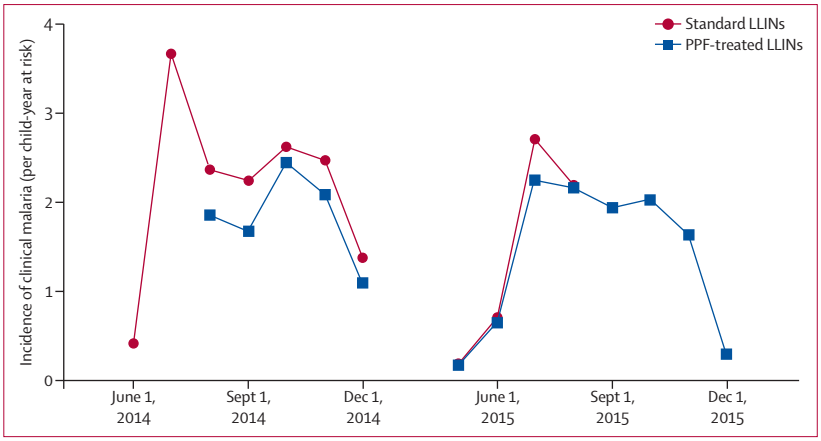

Figure 4: **Effect of PPF-treated LLINs versus standard LLINs on incidence of clinical malaria over time**
PPF=permethrin and pyriproxyfen. LLIN=long-lasting insecticidal net.

3090 (99%) of the planned 3123 entomological collections were successful (houses were locked and could not be accessed in the remaining cases). Female *A gambiae* sensu lato were present in 1687 (55%) light-trap collections (table 4). 45 414 mosquitoes were collected, of which 41 548 (91%) were anophelines (including 40 587 [98%] that were *A gambiae* sensu lato) and 3866 were culicines. 13 584 *A gambiae* sensu lato mosquitoes were identified at the species level (table 4).

There was no difference in proportions of *A gambiae* sensu stricto and *Anopheles coluzzii*, the two most common vectors, between groups (OR 1·19, 95% CI 0·94–1·51; p=0·14). Mean proportions of female *A gambiae* sensu lato, parous mosquitoes, and mosquitoes with sporozoites were lower in the PPF-treated LLIN group than in the standard LLIN group (table 4). The entomological inoculation rate was 42 (95% CI 32–52) infective bites per season in the PPF-treated LLIN group versus 85 (63–108) infective bites per season in the standard LLIN group (rate ratio 0·49, 95% CI 0·32–0·66; p<0·0001; table 4).

The entomological indices were strongly seasonal (figure 5), with a wet-season peak in vector densities each year and an increase in proportions of parous mosquitoes at the end of the wet season. The seasonal pattern in proportions of mosquitoes with sporozoites differed between 2014 and 2015, and patterns of estimated entomological inoculation rates followed trends in vector densities. Because of the rollout of PPF-nets during the 2 year trial, average values for the entomological indices, which were calculated without accounting for the differential representation over time of the two arms of

	Number of malaria episodes reported		Years of exposure		Incidence of clinical malaria (per child-year at risk)		% reduction	Rate ratio (95% CI)	Model-based rate ratio (95% CI)*
	Standard LLINs	PPF-treated LLINs	Standard LLINs	PPF-treated LLINs	Standard LLINs	PPF-treated LLINs			
June, 2014	33	..	79	..	0·4	..	..	..	..
July, 2014	454	..	123	..	3·7	..	..	..	..
August, 2014	244	43	103	23	2·4	1·9	22%	0·78 (0·54–1·13)	0·83 (0·59–1·17)
September, 2014	177	66	79	39	2·3	1·7	25%	0·75 (0·55–1·01)	0·82 (0·60–1·10)
October, 2014	212	155	81	63	2·6	2·5	7%	0·93 (0·75–1·16)	0·93 (0·74–1·16)
November, 2014	193	170	78	81	2·5	2·1	16%	0·84 (0·68–1·05)	0·90 (0·72–1·12)
December, 2014	111	92	80	84	1·4	1·1	20%	0·80 (0·59–1·07)	0·85 (0·63–1·13)
May, 2015	15	14	789	82	0·2	0·2	10%	0·90 (0·42–1·94)	0·95 (0·46–1·99)
June, 2015	42	50	59	77	0·7	0·6	9%	0·91 (0·59–1·41)	0·91 (0·60–1·38)
July, 2015	146	223	54	99	2·7	2·3	17%	0·83 (0·66–1·04)	0·79 (0·63–1·00)
August, 2015	64	266	29	123	2·2	2·2	1%	0·99 (0·73–1·34)	0·98 (0·73–1·32)
September, 2015	..	271	..	139	..	1·9	..	..	..
October, 2015	..	337	..	166	..	2·0	..	..	..
November, 2015	..	304	..	185	..	1·6	..	..	..
December, 2015	..	56	..	189	..	0·3	..	..	..
Total	1691	2047	844	1351	2·0	1·5	24%	0·76 (0·71–0·81)	0·88 (0·77–0·99); p=0·04

LLIN=long-lasting insecticidal net. PPF=permethrin and pyriproxyfen. *Poisson model with offset for exposure time (natural log transformed), a random intercept for cluster, month, and health facility as fixed effects, and an interaction term between month and study group to obtain month-specific estimates.

Table 2: **Incidence of clinical malaria in the cohort**

	Survey 1		Survey 2		Survey 3		Survey 4	
	Standard LLINs (n=1681)	PPF-treated LLINs (n=0)	Standard LLINs (n=1799)	PPF-treated LLINs (n=1874)	Standard LLINs (n=1469)	PPF-treated LLINs (n=1984)	Standard LLINs (n=0)	PPF-treated LLINs (n=4100)
Sex								
Girls	829 (49%)	..	903 (50%)	923 (49%)	713 (49%)	958 (48%)	..	2004 (49%)
Boys	852 (51%	..	896 (50%)	951 (51%)	756 (51%)	1026 (52%)	..	2096 (51%)
Age (months)	35 (15·0)	..	41 (15·0)	41 (15·2)	38 (13·6)	37 (14·0)	..	43 (14·4)
Sleeps under a mosquito net	1549/1681 (92%)	..	1793/1799 (>99%)	1867/1874 (>99%)	1411/1423 (99%)	1901/1910 (>99%)	..	3909/3913 (>99%)
Presence of *Plasmodium falciparum* parasites by microscopy*	851/1627 (52%)	..	1096/1761 (62%)	1124/1843 (61%)	604/1388 (44%)	757/1854 (41%)	..	2159/3758 (57%)
>5000 *P falciparum* per µL*	229/1627 (14%)	..	358/1761 (20%)	338/1843 (18%)	172/1388 (12%)	208/1854 (11%)	..	627/3758 (17%)
Haemoglobin (g/L)*	101·5 (13·5)	..	101·4 (13·9)	103·5 (11·7)	104·3 (12·4)	105·5 (11·6)	..	103·7 (10·7)
Moderate anaemia*†	104/1511 (7%)	..	113/1768 (6%)	54/1782 (3%)	62/1420 (4%)	51/1834 (3%)	..	62/3726 (2%)
Severe anaemia*‡	2/1511 (<1%)	..	7/1768 (<1%)	0	1/1420 (<1%)	0	..	0

Data are n (%), mean (SD), or n/N (%). Survey 1 was done in June, 2014, to July, 2014; survey 2 in December, 2014; survey 3 in May, 2015, to July, 2015; and survey 4 in December, 2015. Includes cohort and additional children, but excludes children during the month of and after distribution of the intervention; thus, the number of children in the first survey differs from those in table 1. LLIN=long-lasting insecticidal net. PPF=permethrin and pyriproxyfen. *Secondary endpoint; assessed at survey 2 when the number of clusters in each study arm was equal. †Defined as <80 g/L. ‡Defined as <50 g/L.

Table 3: **Characteristics of children at the cross-sectional surveys, by group**

the trial, were similar for standard and PPF-treated LLINs (figure 5), despite mosquito densities and proportions of mosquitoes that were parous or had sporozoites being lower in the PPF-treated LLIN group than in the standard LLIN group at almost all timepoints in the contemporaneous comparisons (figure 5). Adjustment for the differential contributions at different time periods was important in providing coherent estimates of the effect of the intervention on overall entomological outcomes (table 4).

There were 21 non-serious adverse events in the standard LLIN group and one in the PPF-treated LLIN group (appendix). Adverse events were mostly mild, although eight, all in the standard LLIN group, were of moderate severity. Only six adverse events (five in the standard LLIN group and one in the PPF-treated LLIN group) were related to the study. All adverse events were resolved after treatment or over time. 19 serious adverse events occurred (ten in the standard LLIN group and nine in the PPF-treated LLIN group), of which 13 resulted in

	Unadjusted estimates		Adjusted estimates*			
	Standard LLINs	PPF-treated LLINs	Standard LLINs	PPF-treated LLINs	Effect estimate	p value
Light-trap collections	1248	1842	..	..	..	..
Mosquitoes collected	16785	28629	..	..	..	..
Female *Anopheles gambiae* sensu lato collected	14489 (86%)	25820 (90%)	..	..	..	..
Species composition of *A gambiae* sensu lato†						
Anopheles arabiensis	56/4774 (1%)	148/8810 (2%)	..	..	..	..
Anopheles coluzzii	403/4774 (8%)	1116/8810 (13%)	..	..	..	..
A coluzzii and *A gambiae* sensu stricto hybrids	5/4774 (<1%)	4/8810 (<1%)	..	..	..	..
A gambiae sensu stricto	4310/4774 (90%)	7542/8810 (86%)	..	..	..	..
A coluzzii‡	403/4713 (9%)	1116/8658 (13%)	..	..	OR 1·19 (0·94–1·51)	p=0·14
Female *A gambiae* sensu lato collected per trap§	12 (32)	14 (36)	9·4 (7·7–11·0)	7·3 (6·1–8·5)	RR 0·78 (0·68–0·89)	p=0·0002
Proportion of parous mosquitoes	60% (625/1038)	62% (1364/2198)	69% (64–74)	61% (57–65)	OR 0·69 (0·52–0·91)	p=0·009
Proportion of mosquitoes with sporozoites¶	4% (206/4858)	3% (273/8935)	4·3% (3·4–5·1)	2·7% (2·3–3·1)	OR 0·62 (0·47–0·83)	p=0·001
Estimated EIR (infective bites per transmission season)	..	..	85 (63–108)	42 (32–52)	RR 0·49 (0·32–0·66)	p<0·0001

Data are n, n (%), or % (n/N), unless otherwise specified. LLIN=long-lasting insecticidal net. PPF=permethrin and pyriproxyfen. OR=odds ratio. RR=rate ratio. EIR=entomological inoculation rate. *Models included cluster as a random effect and month and health facility as fixed effects; numbers in parentheses are 95% CIs. †Typing to species level was missing for 85 mosquitoes in the standard LLIN group and 126 in the PPF-treated LLIN group. ‡Data are *A coluzzii* as a proportion of *A coluzzii* (n=403) plus *A gambiae* sensu stricto (n=4310). §Data are mean (SD). ¶ Missing for one mosquito in the PPF-treated LLIN group.

Table 4: **Entomological results**

hospital admission and six in death; none were related to the study. There were no indications of any serious adverse events associated with the PPF-treated LLINs.

Discussion

In a rural area of Burkina Faso with high bednet coverage, high levels of malaria transmission, and the presence of vectors highly resistant to permethrin, we found that children sleeping under PPF-treated LLINs were 12% less likely to have clinical malaria and 52% less likely to have moderate anaemia than were those sleeping under standard LLINs. The power calculation for the primary endpoint was conservative and based on assumed rates of malaria transmission that were considerably lower than found during the trial. The absolute reduction in clinical incidence was similar to that for which the trial was powered, but the effect size was only 12% (compared with 25% in the power calculations) because the control incidence was higher than anticipated. Clinical incidence saturates with transmission rate, so a 49% reduction in the EIR translates into a lower effect on clinical incidence at a high background EIR. The reduction in moderate anaemia is clinically relevant because anaemia due to malaria is a major cause of mortality in children younger than 2 years.[26]

The entomological data suggest that protection arose from the mass killing of malaria vectors, reducing the density of the vector population, and support evidence of a protective effect for PPF-treated LLINs. In clusters provided with PPF-treated LLINs, vector numbers were reduced by 22% and the odds of finding parous (older) mosquitoes by 31%, consistent with the anticipated mass effect of this intervention. The reduction in vector numbers and fewer older mosquitoes resulted in a 51% reduction in the entomological inoculation rate in the PPF-treated LLIN group.

There was no measurable effect of PPF-treated LLINs on malaria parasite prevalence, which was consistent with other studies that showed that prevalence is only weakly associated with entomological inoculation rate in the observed range of roughly 40–80 infective bites per year in unprotected individuals.[27] The high parasite prevalence of around 60% diagnosed by microscopy, despite levels of bednet ownership being much higher than reported for most countries,[2] probably means that nearly every child in the study area is infected with parasites.[28] Presumably, most children were harbouring infections that had persisted since before the trial and which, therefore, could not have been averted by the new nets. This high prevalence also led to a downward bias in the estimate of effectiveness against clinical malaria because the numbers of clinical malaria cases recorded in both groups of the trial were inflated by cases of fever resulting from non-malarial causes but diagnosed as malaria because of incidental parasitaemia, suggesting that the true efficacy against disease is likely to be higher than 12% (appendix).

The mode of action of the PPF-treated LLIN is complex because it relies on a combination of factors: an intact bednet correctly used provides a physical barrier against blood-seeking mosquitoes, the permethrin on the net has excito-repellent properties that result in rapid knockdown and death of pyrethroid-susceptible mosquitoes, and pyriproxyfen reduces both adult longevity and reproductive outputs of female mosquitoes. Although the permethrin concentration on the two net types was the same, the bleed rates (the rate at which permethrin leaks from the fibres onto the surface of the netting) were greater in the PPF-treated than in the standard LLINs.

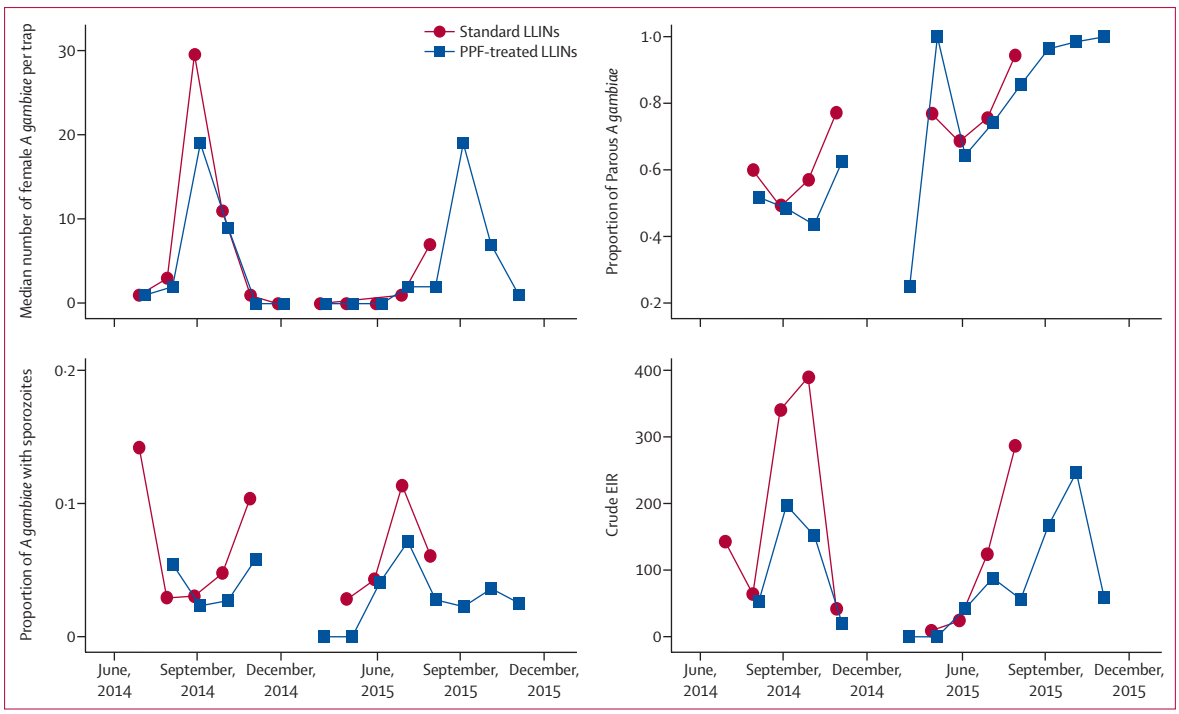

Figure 5: **Effect of PPF-treated LLINs versus standard LLINs on entomological indices**
PPF=permethrin and pyriproxyfen. LLIN=long-lasting insecticidal net. *A gambiae=Anopheles gambiae*. EIR=entomological inoculation rate.

This higher bleed rate meant that the surface concentration of permethrin was probably higher on the PPF-treated LLINs than on the standard LLINs. This hypothesis is supported by studies[10,18,29] that have found that mortality is higher in pyrethroid-resistant mosquitoes exposed to PPF-treated LLINs than in those exposed to conventional LLINs. Another cluster-randomised trial[30] is ongoing in the same district to compare the durability of PPF-treated LLINs and standard LLINs. The results of that trial might enable disentangling of the factor(s) that account for the superior protection provided by PPF-treated LLINs. At present, we cannot be certain whether the protective effect is due to increased concentrations of permethrin on the fibre, PPF, or both.

Few opportunities exist to manage insecticide resistance in *Anopheles* mosquitoes in sub-Saharan Africa, where pyrethroid-treated LLINs are often the only tool for malaria prevention. Nets treated with mixtures of active chemicals, such as Olyset Duo, are an advancement on pyrethroid-only nets, providing that they are cost-effective and there is no cross-resistance between the two chemical classes. Evidence suggests that the elevated concentrations of cytochrome P450s in pyrethroid-resistant *Anopheles* populations might reduce the bioefficacy of pyriproxyfen.[29] Thus, the exceptionally high levels of permethrin resistance in the study site might reduce the effectiveness of both components of the PPF, and the effectiveness of PPF-treated LLINs might be greater in areas with lower levels of pyrethroid resistance and vectorial capacity. Future studies should test, in areas of high pyrethroid resistance, nets treated with mixtures of active ingredients that have different modes of action and which do not include pyrethroids.

This study has two main limitations. First, although the communities were masked to the interventions, it is possible that study participants would view a new net as better than an old one, potentially resulting in under-reporting of clinical cases in the PPF-treated LLIN group. Second, the large-scale use of PPF-treated LLINs might have reduced the number of malaria vectors dispersing from these clusters into adjacent clusters with standard LLINs, and this edge effect will be explored in a future analysis.

The rapid spread of pyrethroid resistance in African vectors is a cause of concern. Although direct evidence that this spread of resistance is interfering with malaria control is lacking, it is likely that it will become a problem in the future. The only strong indication that resistance is interfering with malaria control comes from a two-group trial[31] of pyrethroid nets, which found that addition of piperonyl butoxide to the nets improved protection against clinical malaria.[31] Piperonyl butoxide is a synergist that potentiates the insecticidal activity of permethrin, but cannot fully restore susceptibility in all resistant populations. Therefore, alternative strategies for dealing with insecticide resistance are urgently needed. We selected Olyset Duo as the most commercially advanced, dual-action mixture net and did the trial in one of the most challenging settings in sub-Saharan Africa, where pyrethroid resistance in vectors is high and extremely

common, and vectorial capacity is one of the highest in the region. We found that substantial numbers of malaria cases in children could be averted by these nets in such areas. In Burkina Faso, 6 014 021 uncomplicated malaria cases in children younger than 5 years were recorded in 2017;[3] thus, a 12% reduction in malaria incidence due to use of PPF-treated LLINs would equate to 721 683 cases averted.

Contributors

SWL, N'FS, MP, TS, HR, and ABT conceived and designed the study and drafted the manuscript. BF, MP, TS, and SWL planned the statistical analysis. MP generated the random allocation sequence that assigned participants to the interventions. AO, DO, and SC led the field implementation of the study. ECB supervised the storage and distribution of nets. SS implemented the quality assurance plan for the trial. AD and INO were responsible for the clinical laboratory assessments. N'FS and MWG coordinated the entomology. MWG led the entomological analysis and NG did the insecticide bioassays. ZAO led the data management team. BF, FV, and TS did the statistical analyses. All authors read and approved the final manuscript.

Declaration of interests

We declare no competing interests.

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